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EVALUATION OF THREE APPLICATION RATES OF <u>BACILLUS THURINGIENSIS</u>: EFFICACY AND DEPOSIT ANALYSIS

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Foreword

This report is published as a part of the USDA Forest Service program to improve aerial application of insecticides to eastern broadleaved forests. The program is supported through the efforts of the Northeast Forest Aerial Application Technology Group (NEFAAT). This group is composed of members from the USDA: Forest Service, Northeastern Area State and Private Forestry, and Northeastern Forest Experiment Station; Animal and Plant Health Inspection Service (APHIS), Gypsy Moth Methods Development Laboratory, and Aircraft and Equipment Operations; Agricultural Research Service; and the Department of Entomology, Pennsylvania State University. The Group conducts field and laboratory studies to solve common problems associated with the application of microbial and chemical insecticides. The Group also provides technical assistance in conducting training sessions to improve the quality of operational aerial application programs.

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Introduction

Commercial preparations of Bacillus thuringiensis Berliner var. kurstaki (Bt) are aerially applied as part of the Federal/State/County Cooperative Suppression Program to approximately 0.2 million Ha (0.5 million acres) per year in the eastern United States to suppress potentially defoliating populations of the gypsy moth. In the mid 1980's, most cooperators standardized operational use of various formulations to one application of Bt at a dose of 20 to 40 Billion International Units (BIU)/Ha (8 to 16 BIU/A) and diluted with water at an application rate of 9.35 liters/Ha (1 gal/A). Nevertheless, there was developing interest in using undiluted formulations of Bt in an effort to maximize deposit on the foliage, extend the area of treatment per aircraft load, and minimize costs associated with mixing equipment and support personnel.

The objectives of this study were to determine the effect of one dose of Bt applied at three different application rates on: (1) preventing defoliation, (2) reducing populations, and (3) maximizing deposit on foliage.

Material and Methods

Plot Selection. The study area was located near Martinsburg, WV. It was composed of a mixed oak stand and infested with a building gypsy moth population. Egg mass densities were between 1480 and 4940 egg masses/Ha (600 and 2000 egg masses/A). Twenty-four plots, 20 Ha (50 acre) each, were established for treatments and untreated controls. Each treatment and control were replicated six times. A randomized complete block design was used, with egg mass density as the blocking factor.

Formulation and Dose. The Dipel 8AF® (Abbott Laboratories, North Chicago, IL) formulation of Bt was applied at 50 BIU/Ha (20 BIU/A) using three different application rates: undiluted at 2.9 L/Ha (40 fl oz/A), diluted with water at 9.35 L/Ha (1 gal/A), and at 18.7 L/Ha (2 gal/A). Brilliant Sulfaflavine (BSF) (Organic Dyestuffs, East Providence, RI) was used as the tracer for deposit assessment. Bond sticker (Loveland Industries, Greeley, CO) was added to the formulations at a 2% (v/v) concentration.

Application Methods. In an effort to minimize variation caused by differing meteorological conditions at the time of application, the three application rates were applied simultaneously with three Cessna C-188 AgTrucks, supplied by USDA, Animal and Plant Health Inspection Service, Aircraft and Equipment Operations, Mission, TX. Also, two nozzling systems were used in an effort to generate similar droplet spectra for the three application rates. The application parameters for each aircraft are listed in Table 1. Applications were made at 15 m (50 ft) above the canopy at a speed of 185 km/Hr (115 mph) for two aircraft equipped with flat fans and at 193 km/Ha (120 mph) for the aircraft equipped with micronairs using a 23 m (75 ft) swath width. All of the treatments were a single application of Bt with a total of three days required to complete all treatments.

Population Estimation. Gypsy moth egg mass density was estimated using the Fixed and Variable Radius Plot method (Wilson and Fontaine 1978). Twenty sampling subplot prism points were located within the center 4 Ha (10 A) of each plot. Egg masses were counted on live overstory trees selected using a 20 basal area factor wedge prism and within a fixed 2.6 m (8.3 ft) radius circle at each prism point. Population changes were estimated by counting all egg masses on the ground, debris, and understory trees within the circle in

January 1988, and again in November 1988. New egg masses were distinguished from old ones by feel and by color difference.

Egg Mass Quality Determination. Ten randomly selected egg masses were collected from each plot in March and returned to the laboratory where they were held individually at 27°C and 55% RH for at least three weeks to determine percent hatch and viability.

Defoliation Estimation. The 20 subplot prism points used in estimating egg mass populations were also used for estimating defoliation. Defoliation on all host trees was estimated visually in 10% increments seven weeks after Bt application within the visible canopy in the immediate area of each prism point. The estimates of all points were averaged to obtain a single value for each plot.

Leaf Collection and Tracer Analysis. Two plots of each of the three application rates were sampled for deposit analysis, one replicate plot on each of the two days of spraying. Trees were selected randomly from 15 of the 20 subplot prism points on the first day of sampling and 12 subplot prism points on the second day. Tree climbers were used to remove two branches each from the upper and lower canopy. Fifteen leaves were then selected from each branch, making a total of 30 leaves for each level. Five of these leaves were photographed under long wave UV light (ca 420 nm) using Fujichrome ISO 100 slide film. The remaining 25 leaves were kept at 4°C, their surface areas measured, and analyzed through volumetric tracer washoff techniques to measure mass recovery. The fluorescent tracer was recovered from each leaf by shaking for 25 minutes with 75 ml water. Tracer concentrations were measured fluorometrically in the eluants using a Turner 112 Fluorometer (Sequoia-Turner Corporation, Mountain View, CA).

Image analysis. An Optomax V image analyzer (ITI, Burlington, MA) fitted to a microscope was used to measure the sizes of the photographed droplet stains (Yendol et al 1990, Bryant & Yendol 1991). The spread factor which enables the image analyzer to convert stain diameters to droplet diameters was determined by measuring the sizes of a range of monosized droplets sprayed on adjacent 50% expanded leaves and petri dishes containing silicone oil, in which droplets maintain their spherical shape.

Image analysis results were expressed in terms of: (1) nl/cm^2, which represents the volume of deposit (both volatile and non-volatile components) per cm^2 area of leaf; (2) number of drops/cm^2, droplet density which represents the total number of droplets of all sizes impinging on a cm^2 area of leaf; (3) $D_{VO.5}$, volume median diameter, which represents the drop diameter with 50% of the volume above and below that value, and (4) ($D_{VO.9}$-$D_{VO.1}$)/$D_{VO.5}$) droplet spectrum span, a non-dimensional index based on the range of droplet sizes between the 10 and 90 percentiles divided by the 50 percentile.

Volumetric tracer washoff. Although the image analytical technique does make accurate estimates of the volume of deposit recovered from a spray, it does not measure the mass of non-volatile material in the deposit. Therefore, in an effort to estimate the active ingredient we assumed that the non-volatile tracer was partitioned in a fixed proportion to the non-volatile active ingredient. The volumetric technique enabled the estimation of active ingredient to be made irrespective of the volume applied. The results of tracer concentration recovered from the foliage were transformed to international units (IU) of activity based on the known concentrations of tracer in samples taken from the nozzles of each aircraft upon return to the airport.

Statistical procedures. Egg mass data were transformed to square roots ($\sqrt{x}$) for all analyses (Steel and Torrie 1980). Treatment effects were analyzed by analysis of variance (AOV) and differences between treatment trends were analyzed by Tukey's w-procedure (Steel and Torrie 1960). The trend represented the magnitude of change in egg mass density and was expressed as the ratio of the post/pre-treatment density for each plot. Percentage control (i.e. reduction in egg masses/Ha) was calculated by the following modification of Abbotts' formula (1925): percent control = 100(1-T/C) where T and C are the treatment and control trends.

The parameters of volume per unit area, and droplet density were transformed to log base 10 (Yendol et al 1990, Bryant and Yendol 1991) for all analyses. Treatment effects for log (volume/area), log (droplet density), $D_{VO.5}$, and droplet span were analyzed by AOV, using a split plot design. A pairwise t-test comparison was used to test differences between individual treatments. The differences in mean mass deposit among treatments were determined by AOV, using a split plot design, with sub-plots representing the level of leaves within the canopy. The least significant difference (LSD) separation procedure was used to test differences between treatments. Significance for all analyses was reported at $\underline{P} \leq 0.05$ level.

Results

Population Reduction. Mean pre-treatment egg mass densities (Table 2) were significantly different for treatments and blocks, both at $\underline{P} < 0.01$ as indicated by AOV, using a randomized complete block design. A significant ($F=37.9$; $df=5,23$; $\underline{P}<0.01$) portion of the variation was removed by blocking on pre-treatment egg mass density although the mean egg mass density in the 9.35 L/Ha and 18.7 L/Ha plots

remained different from the control plots. The mean ± SD percent egg hatch among treatments was 86.9% (± 10.0) (range of 60.8% to 95.4%) indicating a healthy population. There was no significant difference in percent egg hatch from egg masses collected from either treatment or control blocks. The population remained healthy throughout the season and increased an average four-fold in the untreated control plots (Table 2).

Differences among egg mass density trends were significant ($\underline{P} < 0.01$) between treatments and controls but not between treatments. The populations in the treated plots fluctuated from reductions to increases and the trend averaged a one and one-half fold increase. Only 4 of 6 of the undiluted treatment plots, 2 of 6 of the 9.35 L/Ha plots and 0 of 6 of the 18.7 L/Ha plots experienced population reductions. The mean percent uncorrected population reduction for the four undiluted plots was 47% and for the two 9.35 L/Ha plots was 21%. The mean percent control among treatments were not significantly different (Table 2).

Defoliation. Defoliation in blocks treated at all three application rates was significantly less than that observed in the control, but not significantly different from each other (Table 2).

Deposit Analysis. The deposits intercepted by the forest canopy were analyzed by image analysis to measure droplet parameters and through tracer washoff to measure mass recovery.

Image analysis

Volume/area. The various measured droplet spectrum data for the different treatments are shown in Table 3. For clarity, the mean volume and mean drops per

unit area are presented as log transformed and untransformed data. A significant difference ($\underline{P}$=0.03) in spray volume per cm^2 of foliage was detected between the 18.7 L(T2) and undiluted treatments (TU). The difference between the 9.35 L(T1) and undiluted treatments was not considered significant ($\underline{P}$=0.08). Slightly more deposit was recovered in the upper than in the lower level of the canopy, 3.00 and 2.34 nl/cm^2, respectively.

Droplet density. There was no significant difference in drop density (drops/cm^2) between treatments; however, differences ($\underline{P}$=0.06) were detected at the canopy level with 3.2 drops/cm^2 at the upper level of the canopy compared with 2.69 drops/cm^2 at the lower level.

Droplet size. There was a significant difference ($\underline{P}$=0.01) in $D_{V0.5}$ between the 9.35 L and undiluted and between the 18.7 L/Ha and undiluted treatments. There were no significant canopy level effects.

Droplet spectrum span. A significant treatment effect ($\underline{P}$=0.02) was found in the mean droplet spectrum span between the 9.35 L/Ha and undiluted treatment, but the difference between the 18.7 L and undiluted was not considered significant at $\underline{P}$=0.08. These data indicate that the undiluted treatment had a slightly wider droplet spectrum than the diluted treatments. There were no significant differences detected between different canopy levels among treatments.

Volumetric tracer washoff. There was no significant difference in the mass of active ingredient reaching the forest canopy among the 3 treatments nor between the upper and lower canopy levels, 54.1 IU/cm^2 and 46.4 IU/cm^2 respectively (Table 4).

The uniformity of distribution of Bt spray in the forest canopy produced by the three application rates was assessed by comparing the standard deviations of the mean log IU/ cm^2 values calculated among averages for levels between trees. The undiluted treatment produced the lowest standard deviation, indicating that the variance of samples from the mean differed least with this treatment (Table 4). The 9.35 L/Ha treatment was the least uniformily distributed as measured by the standard deviation.

Discussion

As expected, the three application rates of Bt provided significant foliage protection. However, the corrected average population reduction pooled for all treatments was insufficient and residual population density was unacceptably high. There were no detectable differences among the treatments based on mean population trends but out of the total six plots where the population reduction trends showed a decrease in population, four were from the undiluted and two were from the 9.35 L/Ha treatments, none were from the 18.7 L/Ha treatment. Undiluted application with the Micronair AU5000 atomizers generated smaller drops than those produced by flat fan nozzles used for the diluted applications. The undiluted treatment also produced the most homogenous deposit distribution. The volumes recovered per cm^2 of foliage for the two diluted applications did not differ significantly from each other but were higher (P=0.08) than that recovered from the undiluted treatment. Nevertheless, there was little difference in the IU's of Bt delivered to the trees among the three application rates.

Both volumetric and image analysis data showed that slightly more material was deposited on the upper level of the forest canopy than in the lower level when estimates

were made based on drop density rather than drop size, which did not vary between the upper and lower canopy for any of the treatments.

In these trials there seems to be only a slight difference between the quality of the deposition obtained with the three application rates. Any difference in biological effects between the treatments would therefore likely be due to active ingredient concentration within the drops, and a slightly better distribution obtained with the undiluted treatment. A better droplet distribution would increase the chances of a larva ingesting Bt while feeding. It is not known if a more concentrated drop density deposit increases Bt efficacy. However, larvae feeding at random on foliage covered with concentrated Bt drops would be more likely to ingest a toxic dose with a small number of leaf bites than on a widely dispersed dilute deposit.

The results indicate that when Bt is applied at 50 BIU/Ha against an increasing gypsy moth population, different volumetric application rates do not appear to influence the foliage protection effectiveness of the Bt. Therefore, it is more cost effective to apply at the undiluted rate than at the 9.35 L/Ha or 18.7 L/Ha application rates as 3 and 6 times the area can be treated per load, respectively.

Further evaluation of undiluted and diluted application rates at higher doses are warranted in an effort to maximize population reduction. These evaluations need to include an assessment of foliage deposit as well as efficacy. Additionally, formulation characteristics (e.g. viscosity, specific gravity) and droplet characteristics (e.g. $D_{V0.5}$) will need to be standardized among treatments in an effort to isolate the most effective dose and volumetric application rate.

Acknowledgements

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Table 1. Summary of application parameters for each aircraft, Martinsburg, WV, 1988.

	Application Rate (L/Ha)		
	2.9	9.3	18.7
atomizer/nozzle	Micronair	Spraying System	Spraying System
model/tip	AU-5000	8004	8008
angle[a]	35°	90°	45°
pressure (KPa)[b]	207	276	276
flow rate (L/min)	22.7	68.9	138.2

[a]The 8004 spray tips were directed 90° straight down and the 8008 directed 45° forward. The AU-5000 blade angle was set at 35°.

[b]30 psi=207 KPa; 40 psi = 276 KPa.

Table 2. Egg mass density (pre and post-treatment) and defoliation estimates for plots treated with Bacillus thuringiensis, Martinsburg, WV, 1988.

Treatment (per Ha)		Egg mass (per Ha)		Trend (post/pre)	Percent Control[a]	Percent Defoliation
dose (BIU)	volume (liter)	pre $\bar{x} \pm$ SEM[b]	post $\bar{x} \pm$ SE	$\bar{x} \pm$ SEM		$\bar{x} \pm$ SEM
20	2.9	1292 (175)	1275 (356)	1.32 (.541)	67	7.5 (1.9)
	9.35	1142 (216)	1725 (259)	1.92 (.526)	53	6.4 (.74)
	18.7	1107 (134)	1877 (336)	1.70 (.248)	58	9.8 (2.2)
Control		1443 (153)	5519 (872)	4.05 (.734)	—	48.9 (8.2)

[a]Percent control = 100 (1-T/C) where T and C are the treatment and control trends.

[b]Mean and standard error of the mean.

Table 3. Summary of the measured droplet spectrum data for the 9.35 liter (T1), 18.7 liter/Ha (T2), and undiluted (TU) treatments, Martinsburg, WV, 1988.

Treatment	Statistic	Volume/[a] cm^2	Drops/[a] cm^2	Volume/[b] cm^2	Drops[b] cm^2	$D_{VO.5}$ (um)	Span
T1	Mean	0.730	0.399	5.37	2.51	204.8	0.501
	Std	0.212	0.063			8.8	0.020
T2	Mean	0.641	0.507	4.37	3.21	171.1	0.515
	Std	0.148	0.044			6.2	0.014
TU	Mean	- 0.101	0.500	0.79	3.16	100.0	0.658
	Std	0.140	0.041			5.8	0.013

[a] Volume per unit area and droplet density are presented as log transformed data.

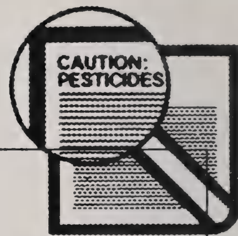
[b] Refers to untransformed means.

Table 4. Summary of mass spray deposit of Bt impacting on sample leaves for three application rates, Martinsburg, WV, 1988.

Treatment[b]	IU[a]/cm² Log Transformed $\bar{x}$ $\pm$SD		IU[a]/cm² Untransformed $\bar{x}$ $\pm$SD	
T1	1.734	$\pm$ 0.419	55.20	$\pm$ 2.62
T2	1.688	$\pm$ 0.349	48.71	$\pm$ 2.23
TU	1.679	$\pm$ 0.217	47.70	$\pm$ 1.65

[a]IU denotes International Units which is a measure of Bt potency.

[b]Treatments included 9.35 L/Ha (T1), 18.7 L/Ha (T2), and Undiluted (TU).



Pesticide Precautionary Statement

This publication reports the aerial application of insecticides. It does not contain recommendations for insecticide use, nor does it imply that the uses discussed here have been registered. All uses of insecticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

Caution: Insecticides may be injurious to humans, domestic animals, desirable plants, and fish or other wildlife if they are not handled or applied properly. Use all insecticides selectively and carefully. Follow recommended practices for the disposal of surplus insecticides and insecticide containers.

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